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Life in Science

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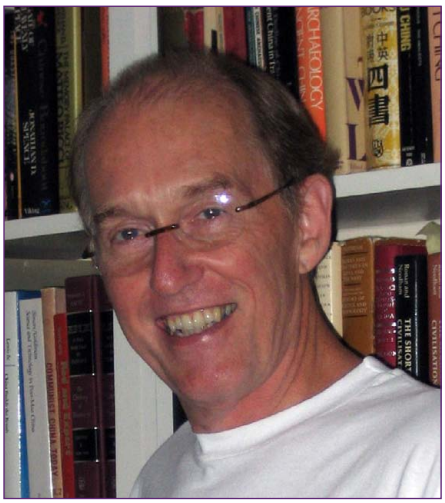

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"What do you do?" The short answer, in the vernacular of the undergraduates, is that I have not yet declared my major. While I answer to the call of "scientist," like most scientists, I am really just a curious person. I am also a materialist seeking material and knowable answers to my curiosity. I think that most curious people, be they artists, poets, musicians, philosophers or scientists, are simply trying to make sense of a puzzling world… different methods, different databases, but similar questions. Scientists, perhaps, have the good sense to cast their work in ways that are not as massively underdetermined as some other approaches. So how have my peregrinations in pursuit of my curiosity led me into a career labeled "scientist?" The underlying theme of this essay is the random and opportunistic nature of my scientific career. Interactions with friends and teachers, unexpected turns of events, and life's myriad contingencies molded my life in science.

When my 7th grade teacher exempted me from the usual arithmetic lessons and gave me a high school algebra book for independent study, a new world of mathematics appeared, and I skipped a year of math which gave me "advanced placement" when I went to college. There, however, suddenly faced with a blank on some form, I impulsively, but presciently, put down "biochemistry" as my field of interest without any idea what that was… simply because I liked biology and chemistry and was finessing the choice between them.

Contemplating a career as a physician (not knowing much about it, however) I expected that I would be a general practitioner, or possibly a specialist of some sort in a small midwestern town, and so I enrolled in the local state university where I would make the contacts with colleagues relevant to my future. The University of Wisconsin was a great place for a curious young student, and I was lucky to have close friends and teachers who nurtured my maturing interests in science.

I took the required "premed" curriculum, math courses, and little else. The exception was several chemistry courses which I took for fun. Math was easy and fun… that is, until my third year when I encountered a grad course in measure theory taught by the renowned Walter Rudin, an experience that convinced me that there was another level of mathematical insight that I did not seem to have. Years later, an eminent mathematician consoled me with the observation that I had run up against a legend and had set a nearly impossible comparison too early in my career.

Two teachers stand out in my memory of my undergraduate years: my freshman English composition teacher, Karl Kroeber, who started at Wisconsin but later chaired the English Department at Columbia University, and the eminent physical chemist, Jack Williams. Kroeber imparted an understanding of clear and coherent writing which has been a lifelong asset. Williams, a specialist in physical chemistry of macromolecules, had a deep but common-sense understanding of chemical thermodynamics that he taught with skill and sympathy. His approach, I think, helped me understand molecules and their behavior in important ways. Later in life I was happy that I could write them appreciative notes before their passing.

I found the traditional medical curriculum of massive busy work both anti-intellectual and rather boring. To remain on campus one summer because of a girlfriend, I took a job in a radiobiology research lab, but by the end of the summer the girlfriend was history and I had enrolled in graduate school for an MS in Radiobiology while I continued part time with medical school.

My first taste of research was to study the ways x-rays can inhibit or cure tumors. This work, under the direction of a young assistant professor, Kelly Clifton, was exciting and gave me my first success in formulating and testing my own ideas. Clifton was an ideal first mentor, young and enthusiastic himself, well-educated in the field, and appreciative of my need for independence. We talked a lot since I shared a small table as a desk in his office. The two years I worked on the tumor bed effect were probably the most important formative influence in my scientific development. I was hooked; I wanted to "do science." The next question was how to make it happen. Two contingencies again intervened: I developed a raging allergy to mice, my experimental system, and the Radiology department could not offer PhD degrees. However, a new faculty member interested in radiation biology had just arrived on campus in the PhD-granting Oncology department, and after a phone call and a brief interview I became a graduate student with Waclaw Szybalski, newly arrived from Rutgers University.

Again, luck was with me. Szybalski and I had the same view of the world. We are both intensely curious rationalists. We do, however, differ in our individual tastes in science. I think there are two kinds of scientists: one kind (me) is interested in understanding how the world works and the other kind (Waclaw) is interested in what use one can make of that understanding… often oversimplified as "basic vs applied" or "science vs engineering."

Szybalski had become interested in the problem of restriction and modification of phages and he persuaded me that I should work on this phenomenon. As a (former) mouse biologist, I had barely heard of bacteriophage, let alone knew how to work with them. I decided to study the fate of the infecting phage DNA by the classical Hershey-Chase blender experiment but failed miserably to get the experiment to work at all. Discouraged, I convinced Szybalski that I should return to radiation and study how x-rays inactivate the function of DNA, comparing the physical damage with the genetic and biological damage. I started with the DNA genetic transformation system in *B. subtilis*, but it was difficult to measure the physical damage in the heterogeneous transforming DNA samples. Most of my PhD research was spent developing DNA strand-break measurement methods for polydisperse DNA samples. Then phage came along.

Bernard Reilly in John Spizizen's lab at the University of Minnesota isolated a series of new phages of *B. subtilis*, which he brought to Madison for characterization in our analytical ultracentrifuge. As one of the local centrifuge experts, I was elected to work with him. Phage φ29 interested me as it was incredibly small; its DNA gave a very homogeneous sedimentation pattern, and it was "infectious." This system, then, was the answer to my problems. I could easily measure physical DNA breakage by sedimentation analysis and simultaneously assess loss of "transfection" as a measure of biological damage. Thus, I traveled to Minneapolis for a week with Reilly to learn how to work with phage. This mini-phage course opened my eyes, finally, to the beauty of phage. I really do believe that scientists develop emotional attachments to their experimental systems, and for me, phage was "it."

In addition to φ29, however, I was investigating physical DNA damage in coliphage T7 as a source of small homogeneous DNA. In time I became the T7 guru in Szybalski's lab where studies on the transcription of phage lambda were hegemonic at the time. In the interval between receiving my PhD (and MD) and moving to MIT for a post doctoral fellowship with Cyrus Levinthal, I followed up on Szybalski's ideas on transcription control in phages by showing that, in contrast to phages T4 and lambda, the transcription of T7 is entirely from one DNA strand, i.e., all the genes are read in the same direction. This result was so clean and beautiful that it cemented my attachment to T7.

Both Szybalski and I thought it good to broaden my experience beyond RNA and DNA, perhaps to protein structure, enzymology, genetics, or physical chemistry. Levinthal had just published some very imaginative ideas on protein structure, a field that I was keen to explore. He was agreeable, and a one paragraph application got me an NSF post-doc fellowship. I was off to Cambridge.

No sooner than I had arrived at MIT than Levinthal announced that he was moving his lab to Columbia in New York. Not only that, but that he suggested that with my knowledge of DNA, RNA and hybridization techniques as well as mouse work, I might investigate the problem of how many genes encoded antibodies. He seemed disillusioned with his ideas for global study of protein structures and so I remained ignorant of this field for several more years.

Although Levinthal offered me a position as an assistant professor of biology at Columbia to run the premed curriculum there, the idea of living in New York, especially on Columbia's uncompetitive salary, led to my immediate search for an academic position, although I had been only a few weeks into my post-doc. At that time, most "good" positions were filled by word of mouth, and the "old school tie" system. One of the few contacts I had made as a graduate student was the chair of biophysics at Yale, Franklin Hutchinson. The year before, as a grad student attending the International Biophysics Congress in Vienna, I had met Hutch on a steamer on the Rhine while on a post-meeting holiday. We spent a pleasant day sailing past the Lorelei and drinking wine. Expecting only some fatherly advice, I was surprised to receive an invitation to visit Yale and give a talk about my recent work. Paul Howard-Flanders, head of the radiobiology section in the Radiology Department, was building a basic research group with a new "center of excellence" grant and apparently he liked my background in radiation molecular biology coupled with my medical background and erstwhile interest in immunology. I went home from the visit with a job offer in hand. After briefly pondering my other options, I accepted Yale's offer and arrived in New Haven in the summer of 1968, a new assistant professor only one year out of graduate school. The generous support of the center grant and the cooperative nature of the group allowed me to be up and running almost immediately. Funds were available for both a technician and a post-doc and I was lucky to recruit a talented research assistant, Ruth Siegel, who had experience in protein purifications that complemented my complete ignorance of this field, and Veronica Maher, a fellow graduate student with Szybalski, who was a great help in getting my lab started.

After a year at MIT struggling to characterize and label immunoglobulin mRNA in myeloma-bearing mice, loaded with ^{32}P, I was ready to return to phage work and the success I had with transcriptional studies on T7. In Levinthal's lab I had learned various forms of gel electrophoresis, a new tool for molecular biologists. Up to this point, all my studies of phage mRNA had relied on nucleic acid hybridizations, so I decided to see what phage mRNA looked like when fractionated by electrophoresis. Bacterial mRNA, at least in bulk, appeared to be a heterogeneous collection of chains being synthesized and degraded with turnover half-times of about 2-3 minutes. I was astounded to find that RNA in T7-infected cells showed numerous discrete sizes, indicating that the phage RNA transcripts were both discrete and stable. This finding provided a new tool to delve further into phage transcriptional regulation, in particular, the "early-late" switch, clearly indentified then only in the T-even phages. In the T-even case, many mutants that were blocked in phage DNA synthesis (D0 mutants) allowed the early functions (seen as protein bands on gel electrophoresis) but did not allow late proteins to be made. This early/late switch was also seen with classes of T-even mRNAs. Since T7 is much smaller and, we believed, much simpler than the T-evens, we set out to solve the mystery of this switch. Why should a block in DNA replication prevent late transcription? Our first experiments with a T7 DNA-negative mutant were rewarding: almost all the usual T7 RNAs were missing. Only one major species was made in the D0 case. Somehow the infectious cycle was blocked at an early stage of mRNA metabolism. The stars were aligned properly for us because just at this point, F. William Studier had completed his first analysis of a set of mutants that nearly saturated the genome of T7. Amazingly, the mutants that blocked late transcription (and DNA synthesis) were in Studier's gene 1, leftmost gene on his linear map.

Serendipity struck again; Richard Burgess, a friend through his wife Ann, who had shared a lab with my wife, Wilma, at Wisconsin, was a graduate student of James Watson at Harvard studying *E. coli* RNA polymerase. Dick was in the process of working out the role of the sigma factor in controlling transcriptional specificity with T4 DNA. Immediately, it seemed imperative to move on to biochemical experiments with T7 DNA and *E. coli* RNA polymerase. Ruth Siegel and I found that in vitro, *E. coli* RNA polymerase would only transcribe the same "early" T7 DNA sequences that we found in the gene 1 mutants in vivo. It appeared that something more than plain *E. coli* RNA polymerase was needed to transcribe the late genes. We speculated (prematurely, it turned out) that the T7 gene 1 product was a new, alternative sigma-like transcription factor. We made one additional observation at this time that we failed to properly appreciate: the late transcription was also resistant to inhibition by the drug rifamycin, known to block *E. coli* RNA polymerase. In another stroke of luck, Jim

Watson arranged for me to present my work at a big meeting on RNA transcription that he was co-organizing in Florence. In my first year as an assistant professor I was on the program of a big-time meeting with hot, new results being championed by Jim Watson.

Unfortunately, my inexperience with proteins caught up with me and I wasted a lot of time making beginner's mistakes. The odd observation of the rifamycin resistance of late transcription led Michael Chamberlin as well as our group to realize that it was not a modified *E. coli* RNA polymerase that transcribed late mRNA, but rather an entirely new enzyme, the T7specific RNA polymerase. We managed to purify and characterize this enzyme after many hours in the cold room. It still amazes me to find that this enzyme, so tricky and labile at first, is now a stock reagent in biotechnology labs.

By the 1970s the once-neglected T7 family of phages was attracting a lot of attention, and I decided to try my hand at working with animal viruses. My wife, Wilma, joined us as a cell culture expert, and we started on the molecular biology of herpesviruses. Herpes simplex virus, we reasoned, was a lot like T4 phage, a large double-strand DNA virus, and in addition it seemed to have medical relevance as the (incorrectly identified) cause of cervical cancer. Work on HSV was much slower than phage work, but there was much to learn. Almost anything we did yielded new knowledge and we had a new tool, restriction endonucleases, just as we had electrophoresis a decade before. Like the large phages, the herpesviruses encode several enzymes of nucleotide metabolism, including a gene for thymidine kinase. This gene gave us the opportunity to study an animal virus gene in much the same way we studied phage genes. Indeed, once we had cloned the HSV TK gene (done under hazmat P4 conditions with Lynn Enquist and George Vande Woude in those old days of recombinant DNA paranoia), we nearly reverted to a phage lab growing only *E. coli* again. This gene proved remarkably useful as a model viral gene, as a target for mutagenesis studies, as a tool for antiviral chemotherapy, and for structure-function studies.

Again, as with the T7 work, the success with HSV attracted new acolytes. I have always been ambivalent about working it a crowded, competitive field, believing that if someone else will do your experiment next week, why not do something else that was not likely to be done by anyone else and make a unique contribution to knowledge? About this time another seemingly random event set me on yet another course. I read an article by Donna Duckworth on the controversy over the discovery of phage in which she noted that one of the discoverers, Fèlix d'Herelle had been at Yale in the 1920s and 30s. One day while waiting to see the dean of the medical school I asked his secretary about old faculty records. To my surprise she pulled out the file on d'Herelle immediately. I was hooked for an hour reading about this strange man, his work on phage therapy, and his mysterious departure from Yale. Thus began my detective work to uncover the real story of the discoverer of bacteriophage, a saga that eventually led to my full length biography of d'Herelle in 1999. This taste of historical research, very much like the work of a scientist, I think, led me to work with my colleague Frederic L. Holmes, to complete his book on the phage genetics of Seymour Benzer, and recently to work on a history of the American Phage Group.

From mathematics, to medicine, on to molecular biology of viruses, and eventually to historical reflections on science, I think my life in science (so far) has been a quest to satisfy curiosity, to provide rational accounts of the world as I see it, and to clarify and organize it for others. It has been great.